Apoptosis, Fibrosis and Senescence

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Abstract

Fibrosis is a major hallmark of progressive kidney disease. The cellular mechanisms that lead to kidney tissue fibrosis are complex and include, for example, increased inflammation, increased oxidative stress, and proximal tubule cell death in the form of apoptosis or senescence. Recent studies have identified TWEAK, a tumor necrosis factor-like weak inducer of apoptosis, as a novel cytokine that mediates kidney inflammation in models of renal fibrosis. Inhibition of apoptosis via TWEAK inhibition has been shown to reduce kidney fibrosis. Recent studies using lineage tracing suggest that interstitial pericytes/perivascular fibroblasts differentiate into myofibroblasts and undergo proliferative expansion during fibrosis. Furthermore, increased expression of nuclear peroxisome proliferator-activated receptor- α in proximal tubules can directly reduce increased expression of transforming growth factor- β 1 and interstitial inflammation in models of renal fibrosis, which suggests preservation of proximal tubule cell metabolism and integrity represents an

important new therapeutic target. In this review, the current evidence and potential molecular mechanisms involved in the development of kidney fibrosis are discussed.

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Introduction

Acute kidney injury (AKI) affects as many as 5% of all patients who are hospitalized and it is now recognized as one of the strongest risk factors for the development of chronic kidney disease (CKD). Unequivocal data have indicated that AKI frequently does not resolve, and even when it does, it can often leave a reduced number of functioning nephrons that subsequently manifest as progressive CKD. It is clear that strategies to prevent AKI, treat AKI, or inhibit the development of CKD as a result of AKI will have a major impact on the incidence of CKD in the community [1]. Currently, only therapies that target the

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surface death receptors that upon activation during renal ischemia activate caspases and initiate apoptosis, which contribute to AKI [6]. This extrinsic apoptosis is a caspase-dependent cell death and hence can be suppressed by pan-caspase chemical inhibitors such as N-benzyl-oxy carbonyl-Val-Ala-Asp-fluoromethylketone or by the overexpression of viral inhibitors of caspases like cytokine response modifier A. Pro- and anti-apoptotic signals converge at mitochondrial membranes, which become permeabilized when the former predominate over the latter. Mitochondrial outer membrane permeability can start at the outer mitochondrial membrane owing to the pore-forming activity of proapoptotic members of the Bcl-2 protein family such as Bak and Bax or can result from a phenomenon (the so-called mitochondrial permeability transition) that originates at the inner mitochondrial membrane due to the opening of a multiprotein complex known as the permeability transition pore complex. In a recent study, Wei et al. [7] examined the role of Bax and Bak in tubular cell apoptosis during ischemic AKI using several gene knockout models. They found that global knockout of Bak protected mice from ischemic AKI with improved renal function. Bax and Bak knockout blocked kidney tubular apoptosis without affecting tubular necrosis. In addition, Bak deficiency prevented mitochondrial fragmentation in ischemic AKI. These studies support a critical role of Bax and Bak in tubular cell apoptosis in ischemic AKI.

Intrinsic tubular cell apoptosis due to caspase-dependent or independent pathways contributes to ischemic as well as nephrotoxic AKI. In this condition, Bax and Bak are activated and form oligomers in mitochondria, which leads to permeabilization of the outer membrane and release of apoptogenic factors such as cytochrome c. Brooks et al. [8] examined the mechanism of mitochondrial damage during AKI. Following ATP depletion or cisplatin treatment of tubular cells, they observed mitochondrial fragmentation prior to cytochrome c release and apoptosis. This mitochondrial fragmentation was inhibited by Bcl-2 but not by caspase inhibitors. Dynamin-related protein (Drp1), a critical mitochondrial fission protein, translocated to mitochondria early during tubular cell injury, and both siRNA knockdown of Drp1 and expression of a dominant negative Drp1 attenuated mitochondrial fragmentation, cytochrome c release, caspase activation, and apoptosis. This study demonstrates a rapid regulation of mitochondrial dynamics during AKI and identifies mitochondrial fragmentation as a novel mechanism contributing to mitochondrial damage and apoptosis in vivo in mouse models of AKI.

Role of TWEAK-Fn14 in AKI and CKD

TWEAK, a TNF-like weak inducer of apoptosis, a type II transmembrane protein and member 12 of the TNF superfamily, was discovered in 1997. Its receptor, Fn14, a type I transmembrane protein and member of the fibroblast growth factor-inducible gene family, was recognized by differential display and later identified in 2001 as the TWEAK receptor. In kidney tissue, Ortiz and his group [9, 10] have presented ample evidence to support the role of TWEAK activation of intrinsic renal cell Fn14 receptors in the pathogenesis of AKI and CKD. Increased expression of TWEAK and Fn14 has been observed in human and experimental models of tubulointerstitial kidney disease. Upregulation of tubular cell Fn14 was seen within 24 h in experimental models of AKI induced by folic acid or by renal ischemia-reperfusion (IR) injury. Kidney TWEAK was also increased in these same models but to a lesser extent than Fn14. Increased tubular Fn14 has also been observed in human ischemic AKI and in acute or chronic human tubulointerstitial inflammation associated with glomerular injury or caused by acute tubulointerstitial nephritis. Targeting TWEAK with neutralizing anti-TWEAK antibodies, the use of TWEAK knockout mice or targeting Fn14 by blocking anti-Fn14 antibodies improved histological and functional features of AKI. Based on the existent evidence of beneficial effects of TWEAK targeting, a clinical trial is exploring the contribution of TWEAK to lupus nephritis. In addition, a potential role of TWEAK in kidney regeneration should be explored.

Senescence and CKD

Cellular senescence is defined as a stable cell cycle arrest elicited in response to multiple types of damage, such as intense oncogenic signaling, DNA damage, and telomere loss [11]. Senescence cells are found in association with aging, tissue repair, and cancer. More recently, programmed cell senescence was documented during mammalian embryonic development. The most widely used senescence marker is senescence-associated β -galactosidase activity, which likely reflects the increased autophagy occurring in senescent cells. Other canonical senescence markers include p53, p21, p16, and reduced RB phosphorylation, which collectively mediate the phenotypic changes associated with cell cycle arrest. A recent study documented the presence of senescence cell death during the development of CKD [12]. These investigators

used young (8–10 weeks) and aged (46–49 weeks) mice and subjected them to 30-min bilateral IR to induce AKI. The aged animals had greater mortality and prolonged elevation in serum creatinine correlating with less epithelial cell proliferation compared to the young. Six weeks after reperfusion, interstitial fibrosis was greater in aged kidneys based on picrosirius red staining and increased extracellular matrix genes. Six weeks after reperfusion, aged kidneys also expressed higher levels of p53 and p21 compared to the young, correlating with greater increases in senescence-associated β -galactosidase, a known marker of cellular senescence. In addition, increased influx of macrophages and CD4 T cells was also seen in aged mice, which was accompanied by increases in mRNA levels of TNF- α and monocyte chemoattractant protein 1. The authors also found reduced microvascular density, correlating with increased nitrotyrosine, a marker of oxidative stress. The findings of this study as well as previous studies [13] suggest that CKD is a chronic condition that promotes cellular senescence and premature aging. This occurs through several mechanisms, including DNA and mitochondria damage, increased reactive oxygen species generation, persistent inflammation, phosphate toxicity, decreased klotho expression, and telomere attrition. Because the recent literature suggests that growth factor signaling has an important role in the aging phenotype, interventions targeting the nutrient-sensing mTOR pathway, such as rapamycin and resveratrol, are of major interest in studies of uremic premature aging. Given the combined role of phosphate and klotho in uremic vascular disease, interventions that target these factors should also be considered.

The Role of Fibrosis in Disease Progression

Recent studies, primarily from animal models of kidney disease, have identified a separate lineage of cells derived from the mesenchyme in the normal kidney that are termed ‘pericytes’ or ‘resident fibroblasts’. These cells represent >5% of normal kidney cells and perform critical homeostatic and regenerative functions, particularly with respect to microvascular homeostasis [14]. In CKD, these pericytes and resident fibroblasts become activated as myofibroblasts. Myofibroblasts are contractile cells that deposit the pathological fibrillar matrix, often described as fibrosis or scar tissue, in the renal cortex and medulla. Myofibroblasts in the glomerulus deposit a pathological fibrous matrix known as mesangial matrix expansion or mesangial nodules, and these contribute to glomerulo-

sclerosis when combined with the loss of glomerular capillaries. Fibrosis is a pathological fibrillar matrix that accumulates in the virtual space between capillaries and tubules of nephrons, or around glomerular capillaries. It obliterates local structures. In the glomerulus, it frequently accumulates initially in the mesangial area, along the capillary loop itself, or in the proliferation of cells that occupy the urinary space, often known as a ‘crescent’. The fibrotic material encroaches on capillaries and prevents them from functioning. Fibrosis per se reduces nephron function and promotes tissue ischemia, leading to distortion of the normal tissue architecture. However, myofibroblasts, the activated pericytes, are contractile and can distort the tissue architecture. The myofibroblast is also an inflammatory cell that secretes innate immune cytokines, chemokines, and oxygen radicals. Furthermore, the myofibroblast can no longer perform the normal functions of the pericyte. Pericytes normally ‘nurse’ capillaries and support microvascular stability. The loss of these functions leaves unstable capillaries that are prone to ineffective angiogenesis, increased permeability, and often capillary demise, known as capillary rarefaction. Therefore, myofibroblasts cause fibrosis, are a source of inflammation, and promote the loss of capillaries. The myofibroblast is therefore a major new target for novel therapeutics in kidney diseases.

Importance of Preserving Proximal Tubule Cell Metabolism during AKI

The renal cortex and, more specifically, the proximal tubule depend heavily on fatty acids as a major source of energy. Fatty acids delivered to renal epithelial cells are primarily used for energy production by mitochondria and peroxisomes. We have described that the process of fatty acid oxidation in peroxisomes and mitochondrial compartments is inhibited in freshly isolated proximal tubules subjected to hypoxic injury and in vivo in the renal tissue of animals subjected to IR injury and cisplatin-induced AKI. This results in the accumulation of long-chain free fatty acids and long-chain acylcarnitines. In addition, fatty acid accumulation during AKI is also accompanied by the accumulation of triglycerides and cholesterol as part of a renal stress response. Recently, we generated proximal tubule peroxisome proliferator-activated receptor (PPAR)- α Tg mice, which showed a protective effect in the proximal tubule by the activation of mitochondrial and peroxisomal fatty acid oxidation in the IR injury and cisplatin models of AKI. In models of

renal fibrosis, using the model of unilateral ureteral obstruction, we demonstrated a significant reduction in proximal tubule cell death and reduced apoptosis accompanied by a reduction in extracellular components in the tubulointerstitial space in PPAR- α Tg mice compared to wild-type mice [15]. These studies suggest that improving proximal tubule fatty acid oxidation represents a novel downstream therapeutic target to ameliorate progression of CKD.

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